



Idiosyncratic responses to drivers of genetic differentiation in the complex landscapes of Isthmian Central America

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Abstract

Isthmian Central America (ICA) is one of the most biodiverse regions in the world, hosting an exceptionally high number of species per unit area. ICA was formed <25 million years ago and, consequently, its biotic assemblage is relatively young and derived from both colonization and in situ diversification. Despite intensive taxonomic work on the local fauna, the potential forces driving genetic divergences and ultimately speciation in ICA remain poorly studied. Here, we used a landscape genetics approach to test whether isolation by distance, topography, habitat suitability, or environment drive the genetic diversity of the regional frog assemblage. To this end, we combined data on landscape features and mitochondrial DNA sequence variation for nine codistributed amphibian species with disparate life histories. In five species, we found that at least one of the factors tested explained patterns of genetic divergence. However, rather than finding a general pattern, our results revealed idiosyncratic responses to historical and ecological processes, indicating that intrinsic life-history characteristics may determine the effect of different drivers of isolation on genetic divergence in ICA. Our work also suggests that the convergence of several factors promoting isolation among populations over a heterogeneous landscape might maximize genetic differentiation, despite short geographical distances. In conclusion, abiotic factors and geographical features have differentially affected the genetic diversity across the regional frog assemblage. Much more complex models (i.e., considering multiple drivers), beyond simple vicariance of Caribbean and Pacific lineages, are needed to better understand the evolutionary history of ICA's diverse biotas.

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Introduction

Isthmian Central America (ICA), including Costa Rica and Panama, is one of the most diverse regions in the globe (Myers et al. 2000). Inserted into the Mesoamerican hotspot of biodiversity, this region roughly covers 0.1% of Earth's land surface, yet harbors an immense number of species. For Costa Rica alone, estimates for some taxonomic groups reach 4–10% of the global biodiversity, and Panama could be even more diverse (reviewed by Bagley and Johnson 2014). Some examples of highly diverse clades in the region include birds (>1000 spp), amphibians (>300 spp), nonavian reptiles (~500 spp), insects (>300,000 spp), and vascular plants (>20,000 spp) (Anger and Dean 2010; Garrigues and Dean 2014; Frost 2019).

ICA has a relatively recent geological origin, although the precise timing of the formation of the region's major landscape features is debated (Montes et al. 2012; O'Dea et al. 2016). Historically, the most accepted hypothesis was that the Isthmus of Panama closed relatively recently,

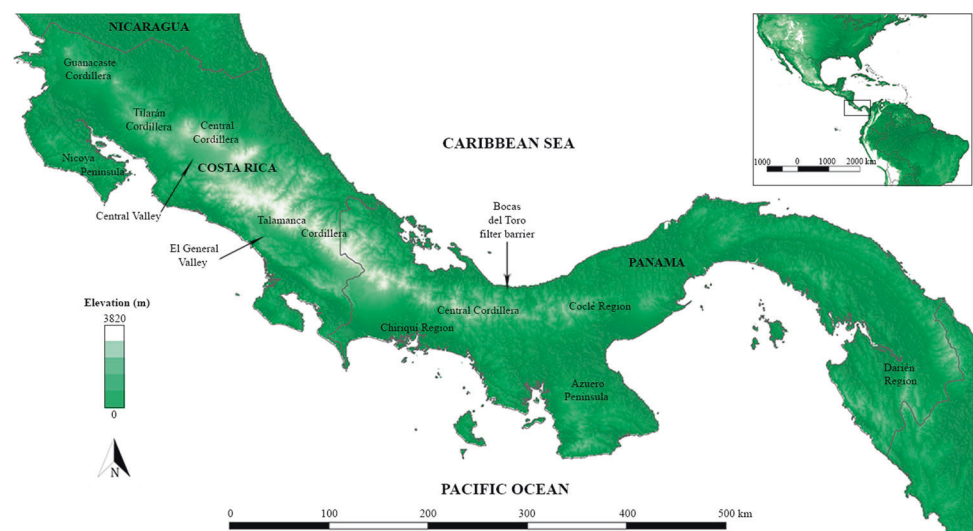
around 3–4 million years ago (Ma) (Coates et al. 1992). Evidence supporting this idea has been based most convincingly on divergence times of marine organisms separated by the Isthmus and several fossils found at the Panama Canal Zone (Keigwin 1978; O’Dea et al. 2016). However, the results from recent studies using petrographic, geochronological, and thermochronological data suggest that the Isthmus of Panama closed much earlier, around 10–15 Ma (Montes et al. 2012). An earlier date is also supported by some fossil evidence (Kirby et al. 2008; Bloch et al. 2016) and molecular phylogenetic studies showing early dispersal between North and South America around 10 Ma (Pinto-Sánchez et al. 2012; Bacon et al. 2015).

Independently of the debate over the precise date of the closure of the Isthmus of Panama, this geological formation facilitated one of the greatest biogeographic events of the Cenozoic, the Great American Biotic Interchange, a bidirectional dispersal of terrestrial mammals between the previously isolated North and South American landmasses (Marshall 1988; Webb 2006). Evidence of intercontinental dispersal in groups such as frogs, birds, and insects also highlights the important influence of the rise of the Isthmus in the assembly of the regional biota (Stehli and Webb 1985; Webb 2006; Weir et al. 2009; Pinto-Sánchez et al. 2012; Wilson et al. 2014). As a consequence, biodiversity in ICA is largely constituted by older northern plus southern lineages that arrived after the completion of the land bridge (Rich and Rich 1991; Savage 2002). Remarkably, the Isthmian fauna also has a strikingly high number of endemics (e.g., Kluge and Kessler 2006; Savage and Bolaños 2009; Bogarín et al. 2013; Garrigues and Dean 2014), including old lineages (Wang et al. 2008a). This high endemism highlights the importance of in situ diversification contributing to the conformation of the regional biota.

The complex tectonic and geological history of ICA resulted in a steep topography that encompasses a wide variety of habitats and climatic regimes over a small surface (Weyl 1980; Gabb et al. 2007). For example, in a span of less than 500 km along the Pacific coast of Costa Rica, the mean annual precipitation varies from ~1800 mm in some areas of the northern dry forest of Guanacaste to nearly 5000 mm in the very humid rainforests of the Osa Peninsula (Holdridge 1987; Bolaños et al. 2005). The physiography of Costa Rica in particular is characterized by NW-trending volcanic cordilleras that can reach altitudes over 3000 m in several peaks (Fig. 1) (Luteyn 1999). Such mountain systems are bisected by valleys that result in a mosaic of sky islands along with steep altitudinal gradients, microclimates, and diverse vegetation zones (Marshall 2007; Bagley and Johnson 2014). In addition, climatic and sea-level fluctuations during the Quaternary (Horn 1990; Islebe et al. 1996) likely promoted species-range contractions and expansions throughout this complex landscape, imposing or removing barriers to gene flow, and potentially playing a central role in the diversification processes (Ramírez-Barahona and Eguiarte 2013; Paz et al. 2019). The climatic dynamics associated with glacial cycles during the Pleistocene are thought to be responsible for shaping the distributional patterns of montane assemblages in the region (Savage 2002; Streicher et al. 2009). Given this historical context, allopatric speciation through vicariant events promoted by physical barriers (Mayr 1942) or parapatric speciation across environmental gradients (Doebeli and Dieckmann 2003) may have occurred extensively in the region, although strong evidence of the latter is still scarce.

Considering its recent geologic history and high levels of richness and endemism, ICA represents an ideal natural laboratory to study the role that landscape features have played in driving the early phases of genetic differentiation.

Fig. 1 Map of the study region including the major topographic features of Isthmian Central America (ICA) and place names mentioned in the text. The relief is drawn over a digital elevation model derived from the NASA Shuttle Radar Topographic Mission (SRTM). The highest elevations in the region are situated in the Talamanca range and overpass 3000 m (Marshall 2007).



One way to understand how such factors have influenced *in situ* diversification in ICA is to study the relationship between geographic or environmental variables and intraspecific genetic divergence. The discipline of landscape genetics combines advances in molecular tools and spatial statistical analyses to assess how the landscape structures populations (Manel et al. 2003; Manel and Holderegger 2013). In the simplest of the models studied under this approach, isolation by distance (IBD), a homogeneous landscape is assumed, and genetic differentiation is expected to increase monotonically with geographic distance due to limits in the per-generation dispersal distance (Wright 1943; Slatkin 1993).

In heterogeneous landscapes, however, dispersal may be more restricted due to the effect of barriers imposed by complex topographies and their associated climatic gradients (Manel and Holderegger 2013). Consequently, genetic isolation is expected to be more closely associated with landscape heterogeneity than geographical distances alone. A model of isolation by resistance (IBR) accounts for the reduced dispersal among populations caused by the relative unsuitability or “friction” presented by heterogeneous environments that may intervene between two populations (McRae 2006). Alternatively, the isolation-by-environment (IBE) model has been recently proposed to describe a process in which genetic isolation between two populations increases with local environmental differences, independently of the resistance or distance imposed by the landscape found between them (Wang and Bradburd 2014).

Quantifying the effects of IBD, IBR, and IBE in promoting genetic divergence within species can aid our understanding of the forces driving genetic diversity, species richness, and endemism in the ICA biota. Moreover, comparing the relative roles of these potential drivers of contemporary genetic variation across multiple codistributed species will reveal common spatial or historical causes behind potentially shared patterns (Feldman and Spicer 2006). As demonstrated in many previous studies, amphibians provide a good model system in comparative phylogeography because they tend to show a marked imprint of the effects of historical, ecological, and geographic factors in driving genetic divergence among populations (Beebee 2005; Smith and Green 2005; Wollenberg et al. 2011; Cruz-Piedrahita et al. 2018).

We hypothesize that spatial genetic variation within co-occurring amphibian species will reflect and reveal similar historical, ecological, and geographic drivers. We predict that species with overlapping distributions will display congruent responses to shared landscape-scale drivers of genetic variation (Avice 1992; Riddle et al. 2000; Feldman and Spicer 2006). Alternatively, variation among species in ecology and life history can result in independent patterns

of genetic structuring in codistributed species (Crawford et al. 2007). In this case, environmental factors will differentially impinge upon species dispersal patterns, creating varied amounts of genetic structure in different species, depending on their habitat requirements (Michaux et al. 2005; Steele and Storfer 2007; Paz et al. 2015). For example, larger species could be less affected by topographic barriers due to their dispersal capabilities, while those species with wider geographic ranges and physiological tolerances could be less affected by climatic variation across the region.

To test this, we compared how landscape features of ICA predict patterns of variation in the 16S fragment within nine amphibian species with overlapping geographical distributions. To this end, we quantified the relative role of isolation by geographic distance (IBD), isolation by resistance due to topography (IBRtopo), isolation by resistance due to habitat suitability (IBRsuit), and isolation by environment (IBE) (e.g., local adaptation) in shaping genetic divergence within each species and tested the following predictions:

- (1) Distance alone (IBD) determines conspecific genetic variation. Linear geographic distance predicts genetic divergence among individuals.
- (2) Prominent mountains are major vicariant barriers (IBRtopo). Dispersal limitation imposed by topographic complexity predicts within-species genetic structure.
- (3) Unsuitable climatic conditions represent dispersal barriers among sampling localities (IBRsuit). Isolation caused by the patchy distribution of suitable habitats in the complex landscapes of the region shapes patterns of conspecific genetic divergence.
- (4) Habitat heterogeneity promotes local adaptation within distinct environments, and thus genetic isolation and divergence among conspecific populations (IBE). Differences among sampled sites in local climatic conditions predict patterns of genetic divergence.
- (5) Codistributed species will show similar relationships between genetic and geographic distance or spatial environmental variation. In contrast, idiosyncratic responses are predicted only to the extent that interspecific differences in life-history traits mediate the interaction between the environment and the organism's dispersal potential.

Materials and methods

Study species

A portion of the DNA sequences used in this study represent new data derived from tissues previously

deposited in the Herpetology Collection of the Museo de Zoología, Universidad de Costa Rica (hereafter UCR). With these samples, we generated an original dataset of mitochondrial DNA (mtDNA) sequences from 350 specimens, corresponding to 95 nominal species sampled from localities across Costa Rica as part of a barcoding survey of Costa Rican amphibians. From this dataset, we discarded potential cryptic species, and selected nine amphibian species with ample geographic sampling and that showed contrasting life histories, including seven anurans and two salamanders, represented by 37 individuals. These species belong to the families Plethodontidae (two species of lungless salamanders), Hylidae (three species of tree frogs), Centrolenidae (two species of glass frogs), Craugastoridae (one species of rain frog), and Bufonidae (one toad species). These species have a variety of habits, reproductive strategies, body sizes, dispersal capabilities, and distribution ranges (Table 1). For example, cane toads (Bufonidae) are bigger, and have large clutch sizes. The two plethodontid salamanders have narrow distributions limited to the Pacific versant of the study region, and are poor dispersers. The direct-developing craugastorid frog species is less dependent on water availability, while centrolenid glass frogs are riparian species, and the terrestrial habitats among the watersheds may act as barriers. These features provide us with a variety of scenarios to test how species with different life histories respond to the same drivers of genetic differentiation.

We supplemented these newly generated data with GenBank data from orthologous DNA sequences for our nine study species, totaling 126 individuals sampled

from Costa Rica and Panama, where we focused the questions of this study. The final dataset combining the newly generated sequences with the public data contained DNA data on a fragment of the 16S ribosomal RNA gene from 163 individuals. In Table S1 we provide species names, GenBank accession numbers, localities, and coordinates for each DNA sequence used in the study. The alignments for these sequences are available at Dryad repository.

While genome-wide markers would be preferable, mtDNA is the single-best standardized marker in multi-species comparative studies that focus on low-level divergence (not polymorphism) where the rapid substitution rate of mtDNA presents a distinct advantage in inferring genealogies, while the conservative structure of the mitochondrial genome provides standardized markers across diverse species (Carnaval et al. 2009; Wang 2013; Paz et al. 2015). Combining these features with the wide availability of public 16S sequence data, our novel nine-species comparative dataset presents a valuable opportunity for testing our hypotheses on the diversification of genetic divergence in tropical tetrapods. While the genealogical information provided by any single locus may be unrepresentative of the genome as a whole, mtDNA in particular has some advantages of single nDNA markers. Being haploid and maternally inherited lowers the probability of incomplete lineage sorting by at least fourfold, if not more (Hudson and Coyne 2002). Also, while gene trees tend to overestimate population divergence times under a model of no migration, mtDNA does so less than nDNA due to the larger effective population size of the former markers (Edwards and Beerli 2000).

Table 1 Amphibian species studied here, with details on life-history traits related to body size (SVL), reproduction, and altitudinal distribution.

Family	Species	SVL (mm)	Reproductive pattern	Clutch size	Elevation range (m)	Total sequences	Sampled localities
Hylidae	<i>Agalychnis callidryas</i>	30–71	Ae/Tpps	11–104	1–1000	51	14
Plethodontidae	<i>Bolitoglossa lignicolor</i>	46–81	Dd	Unknown	0–900	11	9
Hylidae	<i>Dendropsophus ebraccatus</i>	23–35	Ae/Tpps	15–296	0–1300	24	10
Centrolenidae	<i>Espadarana prosoblepon</i>	21–31	Ae/Ts	~20	20–1900	26	12
Plethodontidae	<i>Oedipina alleni</i>	40–58	Dd	Unknown	0–900	5	4
Craugastoridae	<i>Pristimantis ridens</i>	16–25	Dd, Te	Unknown	15–1600	14	11
Bufonidae	<i>Rhinella horribilis</i>	85–175	ETlew	5000–25,000	1–2100	9	8
Centrolenidae	<i>Sachatamia albomaculata</i>	20–32	Ae/Ts	Unknown	0–850	14	7
Hylidae	<i>Smilisca phaeota</i>	40–78	ETspss	1500–2000	0–1000	9	8

This information was obtained from AmphibiO, a global dataset for amphibian ecological traits (Oliveira et al. 2017). The last two columns in the table show the numbers of sequences and localities analyzed for each species.

Dd direct development, Ae arboreal eggs, Te terrestrial eggs, Ts tadpoles into streams, Tpps tadpoles into ponds, puddles, or streams, ETspss eggs and tadpoles into small ponds and shallow streams, ETlew eggs and tadpoles in lotic waters, ETlew eggs and tadpoles in lentic water.

Sequencing methods

For the newly generated data, we carried out DNA extraction on a BioSprint 96 (Qiagen) robotic extractor based on magnetic beads, including digestion with proteinase K (0.4 mg/mL) at 55 °C. We amplified the slowly evolving 16S mitochondrial gene, following Crawford et al. (2010). This gene fragment was amplified and sequenced with primers 16SB-H (aka, 16Sbr-H) (5'-CCG GTC TGA ACT CAG ATC ACG T-3') and 16SA-L (aka, 16Sar-L) (5'-CGC CTG TTT ATC AAA AAC AT-3') (Kessing et al. 2004). PCR products were cleaned using ExoI and SAP enzymes (Werle et al. 1994) with Sanger sequencing products run on ABI 3130 automated sequencers. We performed all enzymatic and sequencing reactions in a high-throughput 96-well format. To confirm base calls, we sequenced the gene bidirectionally. The sequences were aligned using default parameters in MUSCLE (Edgar 2004, <https://www.ebi.ac.uk/Tools/msa/muscle/>) and reviewed by eye.

Potential cryptic species

Preliminary genetic analyses showed that some museum voucher specimens were misidentified, a common mistake in mega-diverse regions such as ICA, where a high number of sister species maintain morphological stasis after speciation (Padial and De La Riva 2009; Funk et al. 2012). After morphological species identification, we confirmed identification of DNA sequences using the Automated Barcode Gap Discovery (ABGD) algorithm (<http://www.abi.snv.jussieu.fr/public/abgd/>), which uses the distribution of pairwise genetic distances between the aligned DNA sequences to identify clusters of DNA sequences that may correspond to named or potential species (Puillandre et al. 2012). This method statistically infers multiple potential barcode gaps or thresholds, and partitions the sequences such that the distance between two sequences taken from distinct clusters is larger than the barcode gap. The method performs well in terms of

speed and success in species identification compared with other DNA barcode algorithms (Paz and Crawford 2012). We processed the 16S alignments in ABGD assuming a Kimura 2-parameter (K2P) nucleotide substitution model (Kimura 1980) and the following settings: prior for the maximum value of intraspecific divergence between 0.001 and 0.1, with ten recursive steps within the primary partitions defined by the first estimated gap, and a gap width of 1.0 K2P is the standard model of DNA substitution for barcode studies performed, as well as other more complex models in identifying specimens (Collins and Cruickshank 2012). Even though there are not many DNA substitution models available in ABGD, species identification success rate is unlikely to be affected by the choice of substitution model (Collins et al. 2012).

To minimize potential taxonomic misidentifications within our nominal species, we confirmed that divergent, conspecific DNA sequences in sympatry showed K2P genetic divergence below 2.5% for 16S. This threshold was conservative compared with thresholds proposed by Vences et al. (2005) and Fouquet et al. (2007).

Estimation of genetic distances and gene trees

We estimated genetic distances in MEGA 4.0 (Tamura et al. 2013) invoking the pairwise-deletion option, thus including sites with gaps when the two individuals being compared had nucleotide data for that site. For each species, we calculated corrected genetic distances assuming the model of evolution determined as the best-fit model of nucleotide substitution for each set of conspecific sequences using MEGA (Tamura et al. 2013). Because we used a landscape genetics approach where the individual sequence is the unit of analysis (Balkenhol et al. 2008), we did not need to identify populations as required for other approaches to estimating genetic divergence, such as F_{ST} (Wright 1951). Details on the sizes of the DNA fragments utilized mean genetic distances, and substitution models for each species are provided in Table 2.

Table 2 Fragment lengths, number of haplotypes, mean genetic distances, and specific substitution models used for tree construction and estimation of genetic divergence in each of the nine species included in this study.

Species	Length (bp)	No. of haplotypes	Mean overall genetic distance	Model
<i>Agalychnis callidryas</i>	1114	50	0.0238	HKY + G
<i>Bolitoglossa lignicolor</i>	496	11	0.0086	T92
<i>Dendropsophus ebraccatus</i>	528	23	0.0064	JC
<i>Espadarana prosoblepon</i>	497	13	0.0071	K2
<i>Oedipina alleni</i>	498	4	0.0248	T92
<i>Pristimantis ridens</i>	487	14	0.0402	K2 + G
<i>Rhinella horribilis</i>	458	7	0.0036	T92
<i>Sachatamia albomaculata</i>	427	8	0.0214	K2
<i>Smilisca phaeota</i>	539	6	0.0236	K2

Gene trees were generated separately for each species using the maximum likelihood (ML) method implemented in the RAxML program, version 8.0 (Stamatakis 2014). We used the same substitution models mentioned above. Bootstrap values were calculated with 1000 pseudoreplicates. To delimit species, we used the ML trees as input and applied a Poisson tree process (PTP) model. This method identifies significant changes in the rate of branching in a phylogenetic tree using the number of substitutions, which are expected to be higher between species than within species (Zhang et al. 2013). PTP is a phylogeny-aware method that provides an objective estimation of phylogenetic entities based on branch-length dynamics instead of grouping just considering sequence similarities (Luo et al. 2018). Following a conservative approach, we report as genetic groups those clades with node values greater than 0.01, and ran an analysis of each species using the Bayesian implementation of the PTP model available in the webserver (<http://species.h-its.org/ptp/>) for 100,000 generations, with thinning = 100 and burn-in = 10%.

Estimation of geographic, resistance, and environmental distances

To compare the relative importance of different landscape variables in explaining genetic divergence within each species, we estimated landscape-derived distances, including linear geographic distance (IBD), resistance distances that accounted for topography (IBR_{topo}) and climatic suitability (IBR_{suit}), as well as local environmental dissimilarity (IBE) between all pairwise combinations of localities within each species. To test for IBD, we estimated linear horizontal geographic distances as pairwise Euclidean distances (in km) between the geographic position of each pair of individuals of each species using the R package, raster (Hijmans and van Etten 2010). To test for IBR, we estimated friction layers (derived from topography and environmental suitability) based on a circuit-theory approach conducted in Circuitscape V.4.0 (Shah and McRae 2008). For topography, we created a layer that quantifies local terrain complexity. We used a raster grid of elevation, with a resolution of 30 arcsec (~0.8 km at the equator), to estimate the standard deviation of a set of 25 adjacent cells to obtain a value of topographic complexity for cells of ~5-km resolution (the same resolution used for the species distribution models (SDMs)). This measure describes well the role of mountains as dispersal barriers as it is highly correlated with maximum altitude on each grid cell. Consequently, higher resistance values are associated with major mountain formations (Fig. S1). For environmental suitability, we used the inverse of a suitability derived from an SDM for each species, assuming that areas

with low suitability have higher resistance for dispersal (Wang et al. 2008a, 2008b).

To construct SDMs, we gathered additional occurrence data across the entire distribution of each species, from the Global Biodiversity Information Facility (<https://www.gbif.org/>) and geographic records from the UCR. We generated SDMs using bioclimatic variables available at worldclim.org (2.5 arcmin resolution ~5 km at the equator) for current conditions. We excluded variables after assessing multicollinearity with a Variance Inflation Factor (VIF) analysis using the R package usdm (Naimi et al. 2014). This analysis quantifies the overall correlation of each variable with all others (Stine 1995). Collinearity is present when the VIF for at least one independent variable is large; then, we iteratively remove collinearity among bioclimatic variables by calculating VIF of variables against each other. To this, we used a value of 10 as the cutoff, as used in previous studies (Zuur et al. 2010). We created all models with a general-purpose machine-learning algorithm (MaxEnt) implemented in the R package, dismo (Hijmans et al. 2012), after parameter tuning and evaluation conducted in the R package, ENMeval (Muscarella et al. 2014; see Supplementary material). The performance of different SDM algorithms is contentiously debated in the literature, and different authors use different approaches (Hernandez et al. 2006; Li and Wang 2013). Fortunately, the relative performance of different algorithms is not a central issue for our implementation of SDMs. Rather than building models to predict what variables may determine species limits, our goal is only to generate surfaces of relative suitability for each species to then estimate the degree of isolation between localities. As long as our approach is relatively robust to assumptions, our analysis is not significantly affected by algorithm performance. Moreover, recent evidence comparing ensembles and single-algorithm methods, demonstrates that MaxEnt produces predictions of similar accuracy than those produced by ensembles, but reduces computational time and simplifies interpretations (Kaky et al. 2020).

To test for IBE, we estimated environmental dissimilarity between pairs of sampling localities. For each locality, we extracted the values for the 19 bioclimatic variables available in the Worldclim dataset (worldclim.org) at 30-arcsec resolution (~1 km). In this case, we kept all variables to have a more detailed estimation of the local climates, regardless of collinearity. We estimated Euclidean pairwise distances using the function 'dist' in R, which computes distances between the rows of a multivariate matrix.

Quantifying the relative effects of IBD, IBR, and IBE

We tested for possible associations between genetic distances and each of the four types of distances that we

quantified (IBD, IBR_{topo}, IBR_{suit}, and IBE), using Multiple Matrix Regression with Randomization Analysis (MMRR), which uses randomized permutation to account for the nonindependence among pairwise distances (Wang 2013). MMRR estimates the effect of each variable as beta coefficients, allowing simultaneous comparisons among them. Because all estimated distances are at different scales, we standardized them (mean = 0, variance = 1) to facilitate the interpretation of the results. We performed the MMRR analysis with 10,000 permutations using an R function provided by Wang (2013).

We implemented a Generalized Dissimilarity Modeling (GDM) analysis to further explore how our independent variables may relate to observed genetic differentiation in each species. This is a multivariate technique that allows modeling a single response as a function of distance matrices for any number of explanatory variables (Manly 1986). GDM approach can fit nonlinear relationships of environmental variables to biological variation using I-spline functions (Ferrier et al. 2007). The spline plots provide insights into the total magnitude of biological change (i.e., genetic divergence) as a function of each gradient, and where, along each gradient, those changes are most pronounced. For each species, we fit a GDM using genetic distance as the dependent variable, and as predictor variables the distances quantified with the different types of isolation (IBD, IBR_{topo}, IBR_{suit}, or IBE). We plotted the I splines to assess how magnitudes and rates of genetic differentiation varied along the gradients represented in our independent (predictor) variables. To select the best combination of predictor variables, we used a permutation-based backward elimination (as detailed in Ferrier et al. 2007). This procedure calculates the unique contribution of each predictor to the total explained deviance, and discards the variable that least contributes to fit a new GDM using a reduced set of $n-1$ predictors. We used 500 permutations and repeated the method until all variables retained in the final model made significant, unique contributions to the explained deviance ($P \leq 0.05$). We performed these analyses with the R package, *gdm* (Fitzpatrick et al. 2020).

One potential limitation of the IBD, IBR, and IBE analyses is the use of predictor variables covering both historical and present-day processes. For example, while topographic variation and geographic distances mirror both historical and ongoing processes, habitat suitability estimations reflect contemporary climatic niches. For the latter case, we attenuate this issue by projecting our suitability models to the Last Glacial Maximum (LGM). However, the IBR matrices estimated for present and past time periods were correlated, suggesting relative stability of environments at the regional level (of the ICA) across the extremes

of interglacial and glacial periods (today and the LGM). Therefore, we decided to use only the suitability predictions for current climate conditions. This will facilitate posterior interpretations. Additionally, we tested for collinearity among variables conducting Mantel tests (Table S2).

Results

Intraspecific patterns of genetic differentiation

We found overall intraspecific genetic distances ranging between 0.36% in *Rhinella horribilis* and 4.02% in *Pristimantis ridens* (Table 2). For the two salamander species in our dataset, we found a major genetic break between populations of *Bolitoglossa lignicolor* in the central Pacific region and those extending from the southern Pacific of Costa Rica to Peninsula de Azuero in Panama (Fig. 2). In the case of *Oedipina alleni*, we found major differences between an individual from the Peninsula de Osa and the rest of sampled localities (Fig. 2).

For the direct-developing frog, *P. ridens*, we found support for a clade containing all Costa Rican samples, two divergent clades from the Coclé region, and a clade containing populations from eastern Panama. In the glass frogs (Centrolenidae), we found evidence for genetic divergence between populations of *Sachatamia albomaculata* in the Caribbean of Costa Rica and the Pacific of Costa Rica plus Panama (Fig. 2). In *Espadarana prosoblepon*, we found four clades: one from the Darien region of Panama, a second containing samples from localities near the Panama Canal, a third grouping ranging from El Copé in central Panama to the Pacific versant of Costa Rica (from Tilarán to the southern foothills of Talamanca), and the final clade containing an individual from the Central Valley of Costa Rica (Fig. 2).

Among the hylid species, *Dendropsophus ebraccatus* was represented by samples only from Costa Rica, which showed a major “north–south” genetic split between the southern Pacific plus central Caribbean versus central Pacific plus the northern portion of the Caribbean versant (Fig. 2). In *Smilisca phaeota*, samples grouped into two major clades mostly divided by the Central Cordillera and the Talamanca Cordillera; these clades overlap in distribution in the lower mountain passes between the Tilarán Cordillera and the Central Valley of Costa Rica (Fig. 2). In *Agalychnis callidryas*, we found that Costa Rican samples belong to three clades: one formed by only Pacific samples, a second including mostly Caribbean samples plus two samples from the northern Pacific of Costa Rica, and a third group containing samples from the Coclé region of Caribbean Panama and the southern Caribbean of Costa Rica (Fig. 2). Samples from central Panama formed a fourth

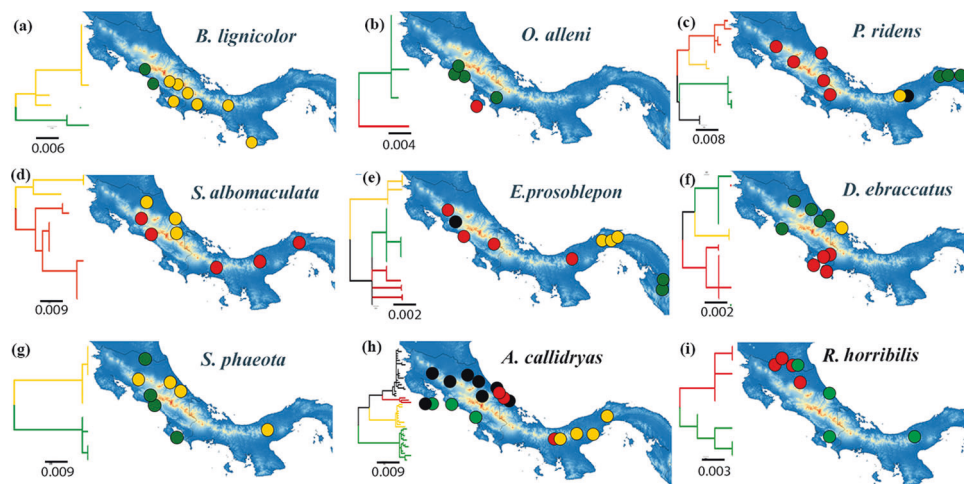


Fig. 2 Phylogeographic structure within each focal species mapped over a topographic map of the study area. Each panel presents the mtDNA gene tree for a given species with major clades, given different colors, mapped to sampling localities (dots) over a digital elevation map of ICA with elevations similar to Fig. 1, but with increasing elevations indicated by warmer colors (sea level: blue; highest peaks: red). Scale bars are gene tree distances given in units of

substitutions/site. Panels (a, b) are salamander species and likely poor dispersers. Panel (c) is a direct-developing frog common in wet forests across the study area. Panels (d, e) are glass frogs mostly associated to riparian habitats. Panels (f–h) are tree frog species that reproduces in ponds and streams. Panel (i) is the cane toad, the larger species in the study and the one with the larger clutch size along with (g). Congruence among species' phylogeographic structure is minimal.

clade. Finally, in the cane toad, *R. horribilis*, we detected two clades that do not show a clear geographic pattern.

Relative role of IBD, IBR_{topo}, IBR_{suit}, and IBE on genetic differentiation

From the MMRR analyses, we found that in five out of nine species, different factors tested here significantly explained the patterns of genetic differentiation, depending on the species (Table 3). None of the models tested explained patterns of genetic differentiation in the salamander species (*O. alleni* and *B. lignicolor*), the hylid frog *S. phaeota*, or the toad *R. horribilis*. In the species where the model significantly explained genetic divergence among populations, we found idiosyncratic responses to the predictors tested. Geographic distance (IBD) had a significant effect on the genetic differentiation in *P. ridens* ($\beta_{\text{IBD}} = 0.648$, $P < 0.001$); topography (IBR_{topo}) explained most genetic divergence for *A. callidryas* ($\beta_{\text{IBR}_{\text{topo}}} = 0.580$, $P < 0.001$) and *S. albomaculata* ($\beta_{\text{IBR}_{\text{topo}}} = 0.580$, $P < 0.001$). Among the factors tested, isolation due to climatic suitability (IBR_{suit}) had the greatest effect on the genetic divergence for *D. ebraccatus* ($\beta_{\text{IBR}_{\text{suit}}} = 0.998$, $P < 0.001$). Local environmental dissimilarity (IBE) was involved in explaining genetic variation within *E. prosoblepon* ($\beta_{\text{IBE}} = 0.486$, $P < 0.001$).

From the GDM results, we confirmed that the response of each species to the factors considered is idiosyncratic, including the magnitude of the gradient of variation of each predictor. We illustrate this heterogeneity by presenting the spline plots for the two variables that better explained the

patterns of genetic differentiation in the species where the model was significant (Fig. 3). Spline plots for all variables tested for each species are available in the Supplementary Material (Fig. S1). Details of the SDMs generated to estimate IBR_{suit} and the respective projections on the study areas are available in the Supplementary Material (Table S3 and Fig. S2).

Discussion

We quantified how different components of the landscape have restricted gene flow and promoted diversification across several amphibian species in ICA. In a majority of the species studied here, at least one of our measures of geographic distance or environmental heterogeneity (i.e., based on topography, habitat suitability, or climatic dissimilarity) significantly explained intraspecific patterns of genetic variation. However, instead of finding a single, major factor driving such patterns across taxa, our results suggest idiosyncratic responses among species, likely related to differences in their life histories. The novelty of our study lies in the generation of new sequences to compile a dataset comprising over 160 individuals of multiple codistributed species and its use in a comparative framework. This is especially relevant in ICA, a highly biodiverse region that has been poorly studied from this perspective (Bagley and Johnson 2014). We test the influence of each predictor of genetic variation (Wang 2013) and quantify the effect size of environmental variation in each predictor on genetic divergence (Ferrier et al. 2007). This was key in our

Table 3 Regression coefficient (β) and significance (p) of MMRR analysis on the association between genetic and geographic distance, topographic barriers, climatic suitability, and environmental dissimilarity for each of the study species.

Species	Full model		IBD		IBRtopo		IBRsuit		IBE	
	β	p value	β	p value	β	p value	β	p value	β	p value
<i>Agalychnis callidryas</i>	0.21	<0.001	0.17	<0.001	0.58	<0.001	-0.33	<0.001	0.05	0.07
<i>Bolitoglossa lignicolor</i>	0.13	0.14	0.25	0.06	-0.25	0.36	0.06	0.84	0.11	0.45
<i>Dendropsophus ebraccatus</i>	0.38	<0.001	0.16	0.08	-0.67	<0.001	0.99	<0.001	-0.10	0.06
<i>Espadarana prosoblepon</i>	0.33	<0.001	0.32	0.13	-0.45	0.05	0.28	<0.001	0.49	<0.001
<i>Oedipina alleni</i>	0.48	0.46	0.63	0.15	0.26	0.59	-0.02	0.96	0.03	0.92
<i>Pristimantis ridens</i>	0.41	<0.001	0.65	<0.001	0.14	0.17	0.02	0.88	-0.16	0.09
<i>Rhinella horribilis</i>	0.18	0.18	0.42	0.02	-0.29	0.59	0.33	0.55	-0.14	0.41
<i>Sachatamia albomaculata</i>	0.68	<0.001	-0.31	0.10	1.17	<0.001	-0.39	<0.001	0.22	<0.001
<i>Smilisca phaeota</i>	0.01	1.00	0.04	0.85	0.10	0.82	-0.04	0.92	0.03	0.87

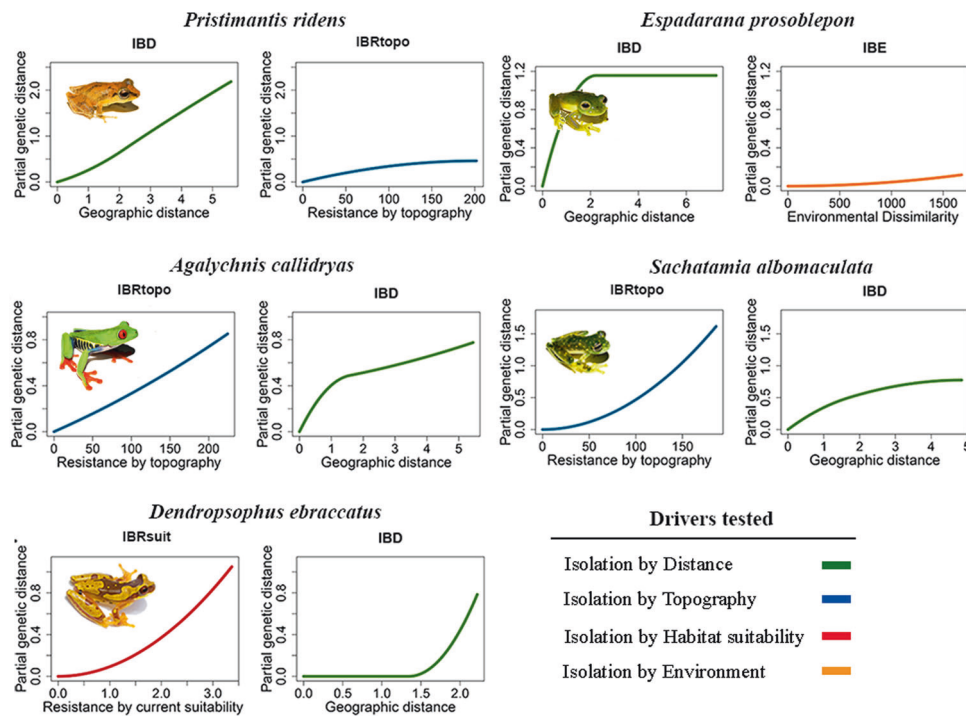


Fig. 3 Generalized dissimilarity model-fitted I splines for the variables that contributed more in explaining genetic differentiation based on the 16S fragment. The figure shows the results only for the five species that the factors tested significantly explained the patterns of genetic differentiation. The maximum height reached by each curve shows the total amount of genetic differentiation associated with the respective variable, holding all other variables

attempt to assess whether genetic structure across all species is similarly influenced by key isolating factors acting in ICA, independently of their temporal reach.

Comparing our results with previous phylogeographic studies of amphibians of ICA, we note that these earlier

studies also show few spatial commonalities among species (Weigt et al. 2005; Crawford et al. 2007; Wang et al. 2008a, 2008b; Robertson et al. 2009). Previous studies have identified at least 31 phylogeographic breaks from mtDNA markers in plants, insects, and vertebrates

(Bagley and Johnson 2014) from across this ~131,000-km² region. Population divergences within the regional frog assemblage have resulted from one or multiple historical events during the Pliocene–Pleistocene (Bagley et al. 2018). Our findings add a new perspective to our understanding of the region's complex evolutionary history by showing that such events are likely driven by multiple forces acting on the ICA and having differential effects on genetic divergence within species. Below, we discuss these results recognizing the strength of the dataset compiled for multiple co-occurring species in a region still understudied in terms of comparative phylogeography, but also bearing in mind the limitations associated with our genetic sampling and the time-scale differences among variables tested.

Geographic distance as the predictor of genetic divergence

Although geographic distance (IBD) was not expected to be the best predictor of genetic divergence due to the region's complex topography and environmental heterogeneity, this variable explained significantly the genetic structure within *E. prosoblepon* and *P. ridens*. Previous work in the region has also found phylogeographic breaks not necessarily associated with obvious physical or climatic barriers (Bagley and Johnson 2014). For example, the Bocas del Toro region is an important filter barrier on the Caribbean coast that separates northwestern from southeastern populations, despite the continuous lowland wet forest habitat connecting Costa Rica and Panama (Crawford et al. 2007; Robertson et al. 2009). This could explain how our samples of *P. ridens* from the Pacific and the Caribbean versants of Costa Rica showed even less divergence between each other than between them and Panamanian samples collected east of the Bocas del Toro region. In this species, Panamanian lineages show long-term geographic stasis in contrast to the rapid geographic expansion that likely occurred from Costa Rica to Honduras (Wang et al. 2008a, 2008b), supporting both the differentiation of Panamanian samples and the absence of structure in the Costa Rican portion of *P. ridens* samples.

Dispersal within streams and among adjacent headwaters can occur in riparian species, such as the here-studied glass frog *E. prosoblepon* (Robertson et al. 2008). Despite the individual's strong site fidelity (Jacobson 2018), their capacity for dispersal maintains genetic homogeneity at the fine scale (2.5–5 km²), including among streams, while terrestrial gene flow naturally decreases with distance (Robertson et al. 2008). Our results support this idea and suggest that geographic distance between watersheds explains genetic differentiation better than topographic or

climatic barriers within a specific watershed, which could be more permeable through riparian corridors for species associated with these habitats.

Major topographic barriers limiting gene flow

The mountainous relief of ICA caused by volcanic and tectonic orogeny (Gabb et al. 2007) may impose a major physical barrier (IBR_{topo}) to the dispersal and gene flow of many species. Intraspecific genetic divergence is expected to be greater in areas of higher topographic complexity (Guarnizo and Cannatella 2013). We found this pattern in the red-eyed tree frog, *A. callidryas*, a lowland species whose genetic divergence is more evident between populations isolated by major orogenic barriers, such as the Talamanca Cordillera, and decreases between northeastern and northwestern populations where topographic barriers are less continuous (e.g., the Tilarán and Guanacaste cordilleras) (Robertson et al. 2009). Similarly, the genetic divergence between individuals of *S. albomaculata* on either side of the central mountain ranges of Costa Rica reflects the importance of mountainous barriers in restricting gene flow for some lowland species. This glass frog is more common below 1000 m (Kubicki 2007), which limits potential dispersal across most mountain ranges in the region, especially across those with continuous elevations above 1500 m from Central Cordillera in Costa Rica to the Tabasará Range of western Panama (aka the eastern Talamanca Cordillera) (Janzen 1991). These results highlight the role of high-elevation Cordilleras as important dispersal barriers for lowland organisms.

In the region, various closely related species distributed on each side of major mountain systems may have experienced isolation promoted by the topography. Some examples include the frogs *Craugastor stejnegerianus* and *C. persimilis* or *Phyllobates lugubris* and *P. vittatus* (Savage 2002; Crawford 2003; Grant et al. 2006), and the bushmaster snakes *Lachesis stenophrys* and *L. melanocephala* (Alencar et al. 2016). These patterns occur even in groups with higher dispersal abilities, such as the birds *Amazilia decorata* and *A. amabilis*, *Cotinga ridwayi*, and *C. amabilis*, or *Manacus candei* and *M. aurantiacus* (Prum et al. 2000; Brumfield and Braun 2001; Stiles et al. 2017). Isolation between Pacific and Caribbean groups is more evident in eastern Costa Rica and western Panama, where topographic barriers become stronger as mountains reach >3500-m elevation in the Talamanca Cordillera (de Boer et al. 1995). In contrast, in Tilarán and Guanacaste cordilleras to the north, where elevations are lower and mountain passes wider (Janzen 1991), the relevance of topography on genetic differentiation might be minor and replaced by other factors such as geographic distance or climate.

Influence of habitat suitability on dispersal and genetic differentiation

Climatic barriers imposed by differential habitat suitability (IBR_{suit}) may shape genetic structure by limiting exchange of individuals and genes among populations. In ICA, such patterns highlight the importance of within-versant climatic variation representing barriers of unsuitable habitats to clades from the central and southern Pacific regions, for example (Holdridge 1987). We found that environmental resistance associated with less-suitable regions best explains genetic differentiation in *D. ebraccatus* (Fig. 3). In this species, our sampling is restricted to Costa Rica, but includes individuals from both versants. Interestingly, we found samples from both the Central Pacific and the Caribbean grouped in the same clade (Fig. 2) that was sister to a clade from the southern Pacific. Previous evidence shows the sensitivity of this same species to climatic barriers in the divergence of lineages associated with the western Panama Isthmus break (WPI, Bagley et al. 2018), a zone characterized by predominantly semiarid conditions since 12–14 Ma (Crawford et al. 2007; Wang et al. 2008a, 2008b). The barrier detected here seems to be associated with the Central Pacific transition between dry and humid conditions, which represents a well-defined boundary for biogeographical provinces of ichthyofauna and herpetofauna (Bagley and Johnson 2014). The region's climatic heterogeneity thus also has an important influence as a driver of genetic differentiation for some representatives of the biota of ICA.

The role of local adaptation in patterns of genetic divergence

Adaptation to local climatic conditions along altitudinal or latitudinal gradients could also promote genetic differentiation in this region. We evaluated climatic heterogeneity (IBE), measuring how different two localities are, independent of the intervening landscape (Wang and Bradburd 2014). Our data show that this factor has a contribution in explaining genetic structure in *E. prosoblepon*. For this species, we detected divergence over short distances, for example, between the Central Valley, the Tilarán Cordillera, and the Central Pacific of Costa Rica.

One of the most striking characteristics of ICA is the variety of climates that converge in such a small area (Coen 1991). For example, transitions between semiarid and very humid conditions occur in a couple of 100 km in the Pacific of Costa Rica, while the temperature may change significantly over shorter distances along elevation gradients in the mountain systems (Coen 1991; Savage 2002). Variation in species-specific tolerances and local

adaptation to such diverse climates influence the distribution of several taxa in the region. Some examples occur even in groups considered good dispersers, such as birds (Garrigues and Dean 2014) and volant mammals like the vespertilionid bats *Rhogeessa bickhami* and *R. io* (Baird et al. 2012). Our analysis revealed a slight contribution of environmental distance (independent of geographic distance) in explaining the genetic structure in this species. Even slight changes in climatic regimes could drive local adaptation and isolation with evolutionary implications such as genetic differentiation for other species in the region.

Additional factors driving phylogeographic structure

Variation in phylogeographic structure among species of anurans can be explained in part by variation in life-history traits (Paz et al. 2015). As shown above, species respond idiosyncratically to each factor tested here, and for a few species, no factor was related to spatial patterns of genetic differentiation. Our full model containing all predictors and their interactions did not significantly explain the genetic divergence in the salamanders, *B. lignicolor* or *O. alleni*, in the masked tree frog, *S. phaeota*, or in the cane toad, *R. horribilis*. Perhaps not coincidentally, for these species, we had lower sample sizes and, consequently, less predictive power (Table S2). Both salamander species are restricted to the Pacific slope of the study region, below 900 m (Savage 2002), which limits the opportunity to test for prominent topographic or climatic barriers along broad altitudinal gradients. However, these species also show several particularities that may account for such a lack of a significant relationship between our predictors and genetic structure.

Although our sampling of *S. phaeota* and *R. horribilis* covered a wide range of climatic conditions and elevations, we did not find evidence of a relationship between the factors tested here and spatial genetic variation in these two frog species. Both species have a large body size that may facilitate high dispersal ability, and they have wide distributions that reflect their broader intrinsic physiological tolerances, even in altered habitats (Savage 2002). These features, along with their high reproductive rate with thousands of eggs per clutch, may explain their distribution and persistence under heterogeneous environmental pressures (Hilje and Arévalo-Huezo 2012; McCann et al. 2014). Thus, barriers such as topography or environmental variation that affect other amphibian species may be more permeable for *S. phaeota* and *R. horribilis*. Our results support the hypothesis that variation among species in life-history traits drives the variable responses among species to a common landscape,

although we must temper this conclusion by the fact that our genetic data came from a single marker. Still, intrinsic factors, such as body size and reproductive mode, may be as important as landscape features in driving lineage diversification (Wollenberg et al. 2011; Paz et al. 2015; Wollenberg Valero 2015).

In conclusion, interspecific differences in life-history traits could be responsible to mediate the interaction between the environment and the organism's dispersal potential. Moreover, the convergence of abiotic factors that may act as drivers of isolation (i.e., complex topography and heterogeneous climates) and the co-occurrence of a heterogeneous biota with disparate life traits may have fueled the increased biodiversity across ICA. Different types of isolation, such as IBD and IBE (reviewed in Sexton et al. 2014) or IBR associated with topographic or climatic barriers (Oliveira et al. 2017a, 2017b), are known to promote genetic differentiation in different organisms. Our study particularly suggests that these forces may act simultaneously even in a relatively small region and differentially affect codistributed species. Certainly, species-specific life-history traits play a key role in how species respond to different drivers of isolation, and such interaction between organisms and their environment must also be considered when trying to understand patterns of genetic divergence (Paz et al. 2015; Rodríguez et al. 2015; Wollenberg Valero 2015). Although based on a single genetic marker, our findings suggest that complex processes, including several abiotic and geographical features, are necessary to explain in situ diversification and understand the evolutionary dynamics of the ICA's amphibian community. We hope that this work motivates the development of further studies, including additional taxa and stronger molecular sampling to continue improving our understanding of the evolution of this region's diverse biota.

Data availability

Data available from the Dryad Digital Repository: <https://doi.org/10.5061/dryad.2jm63xsn0>.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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